The Efficacy of Inoculating Fungi into Conifer Trees to Promote Cavity Excavation by Woodpeckers in Managed Forests in Western Washington[1]

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Abstract

To develop management prescriptions to promote the use of forests by cavity-using wildlife, we have investigated the relationship that wood-decay fungi play in facilitating nest excavation by woodpeckers. We located 78 woodpecker nests in western Washington and documented the presence of basidiocarps and collected wood samples to identify the decay organisms in trees. Basidiocarps of *Fomitopsis pinicola* (red-belted conk) were found on 47.4 percent of all woodpecker nest trees. This fungus was also recovered from wood samples of 7.7 percent of all woodpecker nest trees that did not possess conks. We also identified 32 other fungal types from 20 genera in woodpecker nest trees. We selected *F. pinicola* as the organism to inoculate into trees experimentally to promote colonization by woodpeckers, because this was the most common fungus associated with woodpecker trees and because it met several other criteria. In 1997 and 1998, we inoculated 65 clusters of 10 trees in 34 separate managed forest stands in western Washington. The experimental design of inoculations will allow us to examine the effect of blank (control) vs. fungal inoculations, the influence of tree species (i.e., *Tsuga heterophylla, Pseudotsuga menziesii*), the effect of available snags, and the effect of size/age classes of trees on future colonization by woodpeckers. Visual examinations for presence of mycelia and/or conks, and retrieval of wood samples and isolation of fungal cultures from randomly selected trees (*N*=58) inoculated in 1997 and 1998, indicated the successful colonization of *F. pinicola* in 50 percent to 70 percent of the trees inspected in 1998 and 1999.

Introduction

Most, if not all, cavity-using wildlife are dependent on woodpeckers (e.g., *Picoides* spp.) that excavate cavities (Jackman 1975, McClelland 1977). In western Washington, for example, at least 54 species of birds and mammals require or use tree cavities excavated by woodpeckers (Brown 1985). However, woodpeckers mostly excavate cavities in trees that have decay caused by certain types of

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filamentous fungi, primarily basidiomycetes, that soften the wood of living, injured, and dead trees (Bull and others 1997). Therefore, the interaction between fungi and woodpeckers represents a keystone process that may be necessary to support a robust diversity of wildlife and to complete the complement of vital processes necessary for healthy forest ecosystems.

Traditionally, cavity-using wildlife have been associated with old-growth forests and researchers have suggested or implied that old forests were needed to support these species (e.g., Conner and others 1994). However, because cavities are correlated with old-growth forest, it is not known whether some species of wildlife require mature forests *per se*, or just require a suitable incidence of cavities or snags. The overall objective of this study is to facilitate creation of cavities for wildlife in trees of managed forests in western Washington (see Huss and others 1999).

Specific objectives of this article are to describe the species of fungi associated with woodpecker nest cavities, report on our selection of a fungal species for inoculation into trees to promote use by woodpeckers, describe the protocol for inoculation of a potentially-beneficial fungus into managed forests, and determine the efficacy of treatment for the establishment of fungi.

Materials and Methods

Woodpecker Nest Surveys

During woodpecker nest searches in 1996-1997 we established four general study areas: (1) North Forks, (2) West Forks, (3) Hoh Valley, and (4) Blue Mountain (*fig. 1*, Bednarz and others 1997). In 1998, we searched for woodpecker nests mostly in selected areas in the North Forks and West Forks study areas. The North Forks area was located 6 km north and 11 km west of Forks, Washington, in Clallam County. The West Forks area was relatively small in size (approximately 31 km^2) and was located about 11 km west of Forks, Washington (Clallam County). The Hoh Valley area was approximately 78 km^2 in size and was about 32 km east and 16 km south of the town of Forks, Washington (Jefferson County). The Blue Mountain area covered roughly 93 km^2 and was located approximately 13 km west and 8 km south of Oakville, Washington (Pacific County). The three Forks study sites were primarily dominated by western hemlock *(Tsuga heterophylla)* with secondary dominant tree species consisting of Douglas-fir *(Pseudotsuga menziesii)* and Sitka spruce *(Picea sitchensis)*. Douglas-fir and western hemlock were codominant in the Blue Mountain study area. We incidentally collected data at some woodpecker nests located outside of the described study areas, but these were in ecologically similar environments. All study areas were comprised of state or private lands managed primarily for timber production. Forest habitats consisted of a mosaic of different even-aged forest stands ranging from recently clearcut areas to stands more than 100 yr old. Stands surveyed were generally small, ranging from 10-160 ha in size, and were often bordered by stands which had been clearcut within the last 5 yr.

We conducted nest searches by walking through stands and searching either for woodpeckers or for large snags or partially dead trees with fresh cavities. Fresh cavities were recognized by the presence of recent wood shavings near the base of trees or by recently-exposed wood around the perimeter of the cavity entrance. When a fresh cavity entrance was found, we stayed in the vicinity and watched for

woodpecker activity for 30-60 min. In addition, if woodpeckers were detected during a search, we attempted to follow them to their cavities.

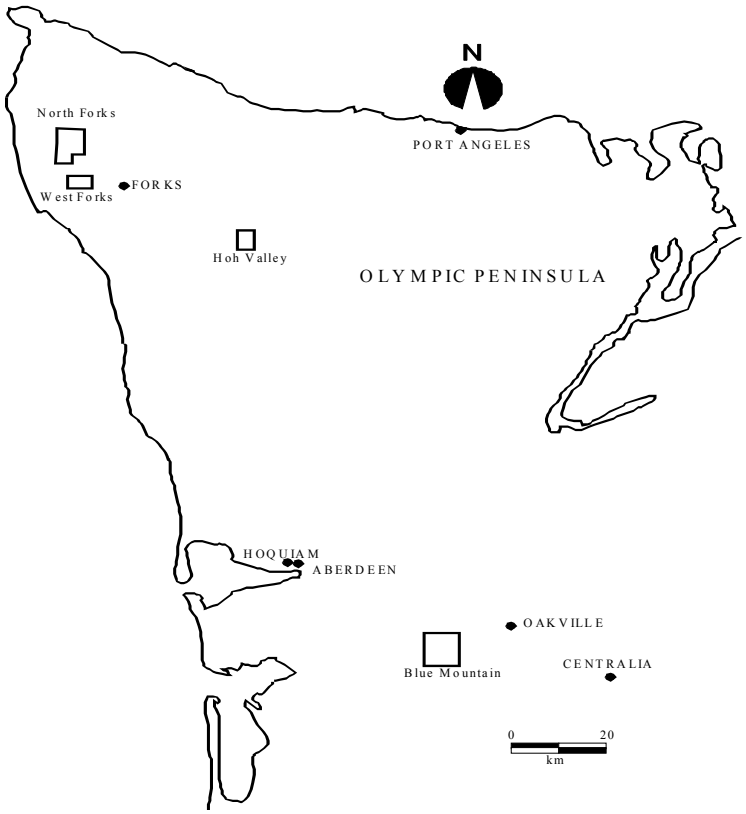

Figure 1—Location of study areas in western Washington. Square and rectangular symbols indicate specific study sites.

Collection of Fungi Associated with Nest Trees

The presence or absence of fungal reproductive bodies or basidiocarps (e.g., conks, bracket fungi) were noted for each nest tree or nest snag. When present, several samples nearest to the cavity entrance were collected. In most cases, we climbed the tree to collect samples. However, when it was unsafe to climb the snag, samples of wood were taken as close as possible to the opening of the nest cavity and conks were retrieved by knocking them off the tree with a pole.

Basidiocarps were air-dried at room temperature to help preserve their appearance and to inhibit degradation. Specimens were stored with Revenge Bug Strips® (Roxide International, Inc., New Rochelle, NY)[5] to prevent pest infestations. Fungal species were identified based on micro- and macroscopic features using standard identification keys (e.g., Arora 1986, Miller 1972, Overholts 1953, Smith and others 1981). Basidiocarps were also examined by Robert L. Gilbertson

[5] Mention of trade names or products is for information only and does not imply endorsement by the U.S. Department of Agriculture.

(University of Arizona) and N. Jay Justice (Arkansas Department of Environmental Quality) to confirm species identification.

Aging of Basidiocarps

Air-dried basidiocarps were weighed to determine their mass and then aged. Many members of the Order Aphyllophorales develop perennial basidiocarps that add a new layer of pores (i.e., spore-producing regions) each year (Alexopoulos and others 1996, Moore-Landecker 1996). The age of the basidiocarp can be determined by counting the number of pore layers established before the actively-growing leading front (1 ridge = 1 yr). In specimens where these outer ridges were less distinct, the basidiocarp was cut longitudinally using a saw. The inner surface was then planed using a scalpel blade so that the number of pore layers could be counted more readily. The age of these perennial basidiocarps indicates when the conk began to form on the surface of the tree, and provides a minimum estimate for how long the fungus was growing on the tree.

Isolation of Fungal Cultures from Basidiocarps

Tissue from the edge of each basidiocarp was removed using a pair of needle-nosed pliers and stored temporarily in a sterile petri dish. After sterilizing with pressurized steam in an autoclave, 20-25 ml of isolation medium (one of two types; Isolation medium #1 and #2; see Bednarz and others 1997) was poured into sterile 100 x 15 mm petri dishes. After the medium had solidified (due to the presence of agar in the culture medium) and cooled, a single fragment of the tissue sample was transferred to the center of the plate using a sterile forceps. These isolation media were nutritionally weak and contained substances to inhibit the growth of fast-growing and potentially contaminating microorganisms while selectively encouraging the growth of a fungal culture from basidiocarp tissue (Tsao 1970). All plates were incubated at room temperature (~24 °C) and checked periodically for the presence of filamentous growth.

Fungi Associated with Wood Samples from Nest Trees

Collection of Wood Samples

Wood samples obtained during the 1996 field season were collected from above, below, and to the right and left sides of the woodpecker cavities and/or at the base of nest trees. Collection of wood samples during the 1997 and 1998 field season was restricted to one or two wood samples obtained directly below the nest cavity and/or at the base of the nest tree. In cases where the wood was less decayed and sound, the sample was removed with an increment borer (5.15 mm diameter). In cases where the wood was too soft to remove with the increment borer, we employed a portable rechargeable electric drill with an 8.00 mm diameter auger drill bit. A hole was drilled 10-15 cm into the tree and particles of wood were collected into sterile plastic collecting bags as the drill bit was withdrawn. Samples were kept refrigerated until we could isolate resident fungi from the wood.

Isolation of Fungi from Wood Samples

Fungi isolated from wood samples collected in 1996 followed the protocol given in Bednarz and others (1997). Culture isolation medium #3 (Bednarz and others 1997) was employed for isolation of fungi from wood samples collected in 1997 and 1998. This medium consisted of a mixture of 20 g of dehydrated malt extract broth, 20 g of agar, 0.2 g of pentachloronitrobenzene (PCNB), and 1 liter of distilled or deionized water. The medium was sterilized by autoclaving at 15 psi at 121°C for 15 min. After cooling to 50-60°C, 2 ml of a filter-sterilized antibiotic stock solution (20,000 units/ml of penicillin and 80,000 units/ml of streptomycin in distilled water) and 1 ml of a benomyl solution (0.1 percent benomyl in 100 percent ethanol) were incorporated into the medium.

Identification of Fungal Cultures

For filamentous fungi and some yeasts, identifications were based on the morphology of cultures growing on malt extract agar and on microscopic characteristics (e.g., reproductive structures, spore morphology, or formation of hyphae). Slide cultures for viewing under the microscope (Larone 1995) were produced by allowing inoculum of the fungal culture to grow on a block of malt extract agar sandwiched between a microscope slide and glass coverslip. Cultures were placed in a moist chamber to prevent the medium from drying out and were incubated at room temperature (24 °C) for several days. After the fungus had grown to the edges of the culture medium onto the surface of the slide and/or glass coverslip, the block was removed and the coverslip transferred to a fresh microscope slide to which a drop of lactophenol with cotton blue dye (Larone 1995) had been added. When enough growth was present, a second slide was prepared by adding a drop of the same mounting medium to the surface of the slide and placing a fresh coverslip over it. The edges of the coverslips were sealed with clear fingernail polish to produce semi-permanent slides.

A variety of literature sources and identification keys were consulted to identify all fungal specimens and cultures to provide some taxonomic category (Arora 1986, Barnett and Hunter 1998, Larone 1995, Miller 1972, Nobles 1948, Overholts 1953, Rayner and Boddy 1988, Rippon 1988, Smith and others 1981, Taylor 1974). Many of the cultures isolated from wood samples were unicellular yeasts. Yeast species are not readily identified based on microscopic characteristics; however, yeasts can be sorted, sometimes to species, based on the outcome of certain physiological tests (e.g., assimilation of various types of sugars). Yeast cultures were tested based on the analytical profile index (API) system for diagnosis of yeasts (diagnostic kits, bioMérieux Viteck, Inc., Hazelwood, MO). We consulted the API data base and identified as many of the yeast species as close to the species level as possible.

Field Inoculations

Selection of Inoculation Sites

We developed an experimental design *(table 1)* to test the effects of tree species, available snags, and size/age classes of trees on the resulting use of inoculated trees by woodpeckers *(table 1)*. This experiment was set up in a hierarchical fashion so we should be able to examine these effects independently and the interaction of these

factors. This evaluation will be done during the next stage of the study to be implemented in 3-10 yr.

Table 1—*Original experimental design for inoculation experiments of Douglas-fir* (Pseudotsuga menziesii) *and western hemlock* (Tsuga heterophylla) *trees.*

Treatments	Presence of snags[1]	Age/size class of trees[2]	Number of clusters	Number of trees per cluster
	Snags absent	Old	4	10
Control		Young	4	10
Group	Snags present	Old	4	10
		Young	4	10
	Snags absent	Old	4	10
Treatment		Young	4	10
Group	Snags present	Old	4	10
		Young	4	10
Total:	2 tree species X 64 clusters X 10 trees/cluster = 640 trees			

[1]Snags absent = < 7 snags (>50 cm dbh)/ha; Snags present = > 7 snags/ha.
[2]Age class designation; young = approximately 50 years old, 30-45 cm dbh; old = approximately 70 years old, 40-60 cm dbh.

Selection of field sites was done with the cooperation of land owners. During this process, we worked closely with representatives from Rayonier, the Washington Department of Natural Resources (DNR), Weyerhaeuser, Port Blakely Tree Farms L. P., I. P. Pacific Timberland Inc., and Hancock Timber Resource Group. Candidate stands for inoculation were required to satisfy the following criteria: supported western hemlock or Douglas-fir as a dominant or co-dominant species and included trees that could be classified as either: (1) relatively small-diameter (<40 cm dbh)/relatively young (approximately 30-50-yr-old trees), or (2) relatively large-diameter (> 40 cm)/relatively old (> 60 yr) stand. We further classified stands into one of two snag-status categories: (1) snags conspicuously present (about 7 per ha), or (2) large snags mostly absent.

In most cases, inoculated and control clusters (each including 10 trees) were located in the same forest stand. In some cases, stands were either not large enough to accommodate two clusters of experimental trees or the structure varied within the stand. In these cases, we located the control cluster in a nearby stand. When experimental and control clusters were placed in separate stands, we made an effort to use stands of similar characteristics (e.g., same dominant tree species, age, and snag density).

Fungi Used in Field Inoculations

Because of the high incidence of *Fomitopsis pinicola* observed associated with woodpecker nest trees during the 1996 field season and its benign nature as a cause of brown rot in wood (e.g., brown-rotter: capable of utilizing primarily cellulose and

hemicellulose in wood but not lignin [Rayner and Boddy 1998]), we chose this organism in our field tests. Three basidiocarps of *F. pinicola,* from three different study locations in western Washington were used to derive mycelial cultures (Bednarz and others 1997). Cultures of these individuals were morphologically indistinguishable from one another, but vegetative compatibility analysis (Huss 1993, Mounce 1929, Worrall 1997) of the these isolates indicated that these represented three genetically-distinct individuals of *F. pinicola* (*i.e.,* basidiocarps no. 36 and 64 from hairy woodpecker *[Picoides villosus]* nests 17 and 36, respectively, and basidiocarp no. 43 from northern flicker *[Colaptes auratus]* nest 22).

Preparation of Wooden Dowel Spawn

Dowel plugs (12.8 cm long) were cut from 2.24 cm diameter hardwood. A 0.96 cm diameter hole was drilled down the center of each dowel using an auger drill bit to create an axial channel (C. G. Parks, pers. comm.). Plugs were soaked in distilled water (1:4 mass to volume - 1 kg of wood per 4 l of water) for 12 hr. Plugs and water were autoclaved (i.e., 121°C at 20 psi) for 30 min. The contents were allowed to cool and were soaked overnight. Excess liquid was decanted off the dowel plugs followed by a rinsing with distilled water. Dowels to be used for the control inoculations were prepared in the same manner but autoclaved a second time for 1.5 hr to insure that any resident microorganisms were killed.

A sterilized grain medium was used in conjunction with dowel plugs to support good mycelial growth and reduce the time necessary for the fungus to invade the wooden substrate. Dry millet and perlite (4:1 by weight) were mixed together, then added to distilled water in a ratio of 1 part by weight of dry ingredients to 1.5 parts by volume of distilled water (i.e., 1 kg of grain/perlite for every 1.5 liters of distilled water). This mixture was autoclaved (i.e., 121°C at 20 psi) for 30 min, then allowed to cool to room temperature and any excess liquid was drained off. Gypsum (2 percent by weight of $CaSO_4 \bullet 2H_2O$) and powdered chalk or whiting (0.5 percent by weight of $CaCO_3$) were mixed into the cooked grain medium to help buffer the pH and to help prevent the grain from sticking together (Elliot 1985, Fritsche 1978).

Plugs were loaded with grain medium by stuffing material into their axial channels. Twenty-five wooden dowels were placed into a square aluminum cake pan (20.3 x 20.3 x 3.7 cm) and 250 ml of malt extract broth was poured over the surface of these dowels. The surfaces of the dowels were lightly covered with grain/perlite medium. The pan was placed into an autoclavable spawn bag with filter patch (Dimensions: 53.3 x 20.3 x 12.1 cm, Fungi Perfecti, Olympia WA) and the end of the bag was sealed with wooden clothes pins. The bag and its contents were autoclaved for 1.5 hr, then allowed to cool. After sterilization the dowels were inoculated with mycelium of *Fomitopsis pinicola* (isolates 36A or 64A) that had been grown previously on 2 percent malt extract agar. Material was incubated at room temperature for 2-3 mo. and growth was monitored.

Inoculation of Control and Test Stands of Trees

Inoculations of trees were made by climbing approximately 8 m (within one standard deviation of the mean height of woodpecker nests) up a Douglas-fir or western hemlock tree and a 2.24 cm to 2.56 cm diameter hole was drilled, 5-8 cm deep using an battery-operated drill. A manually-operated brace was then used to drill out the remaining hole to a depth of 13-15 cm. A blank or inoculated dowel was

inserted into the hole and a piece of PVC tubing (7.70 cm long by 2.56 cm in diameter) was inserted in the hole adjacent to the dowel. About 5 cm of the tubing remained outside the entrance of the hole to prevent the tree from healing over this wound (C. G. Parks, pers. comm.). Holes were made on the north sides of trees, which was where woodpeckers most often excavated their nests (Bednarz and others 1998). Fungal inoculations were made using dowels impregnated with cultures 36A and 64A of *F. pinicola*.

A subset of the trees were inoculated with culture 64A in a similar manner as described above but using dowels of a smaller diameter (1.0 cm and 1.4 cm) with no axial channels drilled through the center. Holes drilled for these dowels were 1.28 cm to 1.60 cm in diameter. After the blank or inoculated dowel (7.7 cm long piece 1.28 cm diameter) was inserted into the hole, PVC tubing was driven 2.56 cm into the hole with a rubber mallet.

Inspection and Sampling of Trees Inoculated in 1997 and 1998

All experimental tree stands that we established in 1997 were visually inspected in 1998 and 1999 to determine if there were any signs of fungal establishment or other changes. In 1998, we randomly selected 10 clusters treated with viable inoculum and collected wood samples from two trees (a total of 20 trees) in each cluster to determine if *F. pinicola* isolates 36A or 64A were established. In 1999, we randomly selected 19 clusters and collected wood samples from two trees per cluster (20 trees inoculated in 1997 and 18 trees inoculated in 1998). Wood cores were extracted from trees using an increment borer (5.15 mm diameter), about 1 cm below the inoculation hole and extending 5-10 cm into the tree. Prior to use on each tree, the increment borer was rinsed in 70 percent isopropyl alcohol to sterilize the surface. Wood cores were placed in sterile plastic bags and kept refrigerated until isolation of fungi could be accomplished. Tree wounds were covered with a pruning sealant to minimize exposure to other fungi.

Isolation of *Fomitopsis pinicola* from Wood Samples

Four isolation plates per wood sample were established using isolation medium #3. Bits or particles of wood were transferred, using a sterile forceps, to the center of a 100 x 15 mm petri dish containing 20-25 ml of the isolation medium. All isolation plates were incubated at room temperature (24 °C) and checked periodically for the presence of fungal growth. After colonies appeared on the plate, a piece was transferred and grown on culture plates containing a 2 percent malt extract agar and incubated at room temperature for a 1-2 weeks. Culture and microscopic characteristics were checked to determine whether the fungus was *F. pinicola* or some other fungal species. Isolation procedures were repeated for wood samples that did not yield this fungus to determine its presence or absence in the collected wood samples.

Vegetative Compatibility Analysis

Isolates of *F. pinicola* were paired with stock cultures of *F. pinicola* (36A, 43A, and 64A) on malt extract agar plates and incubated at room temperature for several weeks. The plates were examined for lines of demarcation that are indicative of vegetative incompatibility and suggest when isolates are genetically different. If no

lines of demarcation were observed between the two paired colonies then this would suggest that isolates represented the same genetic individual. This test was employed to determine if the cultures inoculated into trees represented the same fungus extracted from the tree (Bednarz and others 1997, Huss 1993).

Results and Discussion

Woodpecker Nests

Between 1996 and 1998, we located and collected data from 78 cavities occupied by nesting woodpeckers on or near the study areas *(fig. 1)*. These included 51 hairy woodpecker cavities, 26 northern flicker cavities, and 1 red-breasted sapsucker cavity. We saw and/or heard pileated woodpeckers occasionally but these birds were extremely wide ranging and we did not document nesting. In 1997, we observed a single downy woodpecker in the Blue Mountain study area, but could not locate its nest. While we did occasionally detect red-breasted sapsuckers in the Forks area, nesting activity was only recorded in the Blue Mountain study area *(fig. 1)*.

Fungal Diversity Based on Basidiocarp Data (1996-1998)

Fungal basidiocarps were present on 50.0 percent of the woodpecker nest trees (N = 78). Of the 82 conks collected over the course of 3 yr, 93.9 percent represented *Fomitopsis pinicola* (mean age = 7.9 yr; mean mass = 221.6 g), 2.4 percent represented *Ganoderma applanatum* (mean age = 8.5 yr old; mean mass = 105.8 g), and 3.7 percent represented *Trichaptum fuscoviolaceum* (mean age = 1.3 yr; mean mass = 2.4 g). Among hairy woodpecker nest cavities for 1996-1998, evidence of fungal basidiocarps was present on 54.9 percent of all trees regardless of tree species (N = 51). Basidiocarps were found on 42.3 percent of the northern flicker nest trees (N = 26). The mass and age of the conks from *Fomitopsis* and *Ganoderma* indicate that these fungi were growing in the wood for a substantial period of time before excavation of the tree by woodpeckers. These organisms are completely heterotrophic and obtain all their nourishment from the wood they decay. Consequently, inoculation of trees intended for the purpose of attracting woodpecker and other cavity utilizing species will likely take a minimum of 5-10 yr before colonization occurs.

Wood Samples and Culture Isolations

Of the 211 wood samples collected from nest trees during the summer field seasons of 1996-1998, at least 33 different fungal types representing 21 genera were isolated and identified (N = 471 cultures) (*table 2,* Huss and others 1999). Bacteria represented 2.8 percent of the subcultured microorganisms.

We extracted a wide diversity of fungi from the wood samples, including yeast and filamentous forms. Observations of the mode of sexual reproduction or the presence of microscopic structures unique to a phylum (e.g., clamp connections in members of the phylum Basidiomycota) are often necessary to designate the fungal phylum of a culture (e.g., Oomycota, Ascomycota, Basidiomycota), but this was not observed in most of our cultures. Because we could not distinguish phyla, we classified most isolates of filamentous fungi as belonging to the Form-Phylum Deuteromycota (the asexually reproducing or imperfect fungi) or the Phylum

Zygomycota (based on presence of asexual reproductive structures—sporangia) and discuss these separately from the yeasts.

Table 2—*Fungi and other microorganisms recovered from wood samples obtained from woodpecker nest trees in western Washington, 1996-1998.*

Category or species	Number of samples (N = 471)	Percent occurrence
Aspergillus sp.	7	1.49 pct
Bacteria	13	2.76 pct
Basidiomycete #1	9	1.91 pct
Basidiomycete #2	5	1.06 pct
Basidiomycete #3	13	2.76 pct
Basidiomycete #4	1	0.21 pct
Fomitopsis pinicola	21	4.46 pct
Basipetospora sp.	3	0.64 pct
Cladosporium sp.	15	3.18 pct
Fusarium sp.	28	5.94 pct
Gliocladium sp.	6	1.27 pct
Graphium sp.	1	0.21 pct
Mycelia sterilia	11	2.34 pct
Oedocephalum sp.	1	0.21 pct
Oidiodendron sp.	13	2.76 pct
Oomycete (Saprolegniales)	21	4.46 pct
Paeciliomyces sp.	7	1.49 pct
Penicillium sp.	32	6.79 pct
Rhizoctonia sp.	4	0.85 pct
Trichoderma sp. (large-spored)	42	8.92 pct
Trichoderma sp. (small-spored)	27	5.73 pct
Verticillium sp.	2	0.42 pct
Yeast (unknown)	88	18.68 pct
Candida sp.	36	7.64 pct
Cryptococcus sp.	6	1.27 pct
Kloeckera sp.	6	1.27 pct
Rhodotorula sp.	1	0.21 pct
Torulopsis sp.	4	0.85 pct
Unidentified Filamentous Fungus	12	2.55 pct
Zygomycete #1–*Mucor* sp.	20	4.25 pct
Zygomycete #2–*Mucor* sp.	10	2.12 pct
Zygomycete #3	4	0.85 pct
Zygomycete #4–*Absidia* sp.	1	0.21 pct
Zygomycete #5	1	0.21 pct

Yeasts were the most common single type of fungus isolated (29.1 percent, N = 211). Based on the API system, a number of these sorted together into subgroups. Most yeasts belonged to the genera *Candida*, *Cryptococcus*, or *Rhodotorula (table 2)* which are among the most common indigenous microorganisms found in soil (Atlas and Bartha 1998). Some species of *Cryptococcus* are associated with the dried fecal material and nesting materials of birds (Rippon 1988). Other genera of yeasts observed included *Torulopsis* and *Kloeckera*.

The remaining fungal isolates (67.3 percent) were filamentous, representing at least 27 distinct fungal types, genera, or species. A large number of the samples represented the group deuteromycetes (Form-Phylum Deuteromycota). Filamentous deuteromycetes isolated and identified included the following genera: *Aspergillus*, *Basipetospora*, *Cladosporium*, *Fusarium*, *Gliocladium*, *Graphium*, *Oedocephalum*, *Oidiodendron* (some cultures may represent the genus *Geotrichum* instead; the culture characteristics of both genera are similar), *Paeciliomyces*, *Penicillium*, *Trichoderma* (two types, differentiated by the production of large and small asexual spores [conidia]), *Verticillium*, and several specimens tentatively assigned to Mycelia Sterilia (i.e., vegetative hyphae with no spores evident) (*table 2*). Some isolates were lacking distinct characteristics and could not be identified to a specific category and thus were listed as unknown. Remaining groups included several species belonging to the Phyla Zygomycota, Oomycota, and Basidiomycota. Each group of zygomycetes and basidiomycetes that were differentiated into subgroups (#1, #2, etc.) were distinctly different from one another, but in most situations, were not identified beyond this level.

Of the basidiomycetes observed during this study, the most commonly encountered was *Fomitopsis pinicola*. We recovered cultures from wood samples and/or observed basidiocarps of *F. pinicola* from 55.1 percent of the nest trees examined ($N = 78$ trees). Basidiocarps of this fungus were found in 47.4 percent, and cultures retrieved from wood samples from 15.4 percent of all trees sampled ($N = 78$). Cultures of this fungus retrieved both from wood samples and basidiocarps were found simultaneously on 7.7 percent of the trees sampled. These data suggest that some nest trees without visible conks are also likely to be harboring colonies of *F. pinicola*. Thus, *F. pinicola* was probably present in woodpecker cavity trees more frequently than we were able to document (55.1 percent) due to sampling error during the retrieval and culture isolation process.

Classification of these fungi into major groups indicates that the predominate fungi found in nest trees or snags are filamentous deuteromycetes and yeasts, but these do not necessarily represent the initial invaders and primary agents of decay in the wood. As an agent of decay, *F. pinicola*, which is a basidiomycete typically causing brown rot, produces digestive enzymes capable of removing cellulose and hemicellulose. The wood shrinks upon drying and cross-checking occurs making the wood brittle (Rayner and Boddy 1988); this probably enables woodpeckers to excavate chunks of material more efficiently compared to wood without decay.

One potential tree pathogen was isolated from one wood sample. At a nest site found outside the primary study sites (not shown in *fig. 1*) we isolated a culture (No. 13-1) which was identified as belonging to the genus *Oedocephalum*. One species in this genus represents the conidial (asexual) state of the basidiomycete *Heterobasidion annosum*, a common cause of butt and root rot in conifers (Agrios 1997, Barnett and Hunter 1998).

We recorded from 1-10 distinct types of fungi with an average of 3.4 fungal types per woodpecker nest tree ($N = 78$). The presence of an ensemble of fungi in decaying wood surrounding a woodpecker nest cavity represents a complex community of organisms with the potential for multiple interactions. Some of these species interactions may be intricately related, such that a succession of fungi move from initial invaders through a series of organisms capable of utilizing various components of wood as these become available over the course of time. Some fungi may enhance the ability of other fungi to grow more effectively through the

substratum creating mutualistic associations. Conversely, some fungi may become antagonistic due to the presence of other microorganisms by competing for the same food source. Other organisms may impede the growth of neighboring fungi through the production of waste products or secondary metabolites (e.g., antibiotics, toxins). For example, Atlas and Bartha (1998) and Rayner and Boddy (1988) indicate that species of the soft-rot fungus *Trichoderma* are antagonistic to the growth of other fungi. Additionally, some fungi may exist as parasites or predators on other organisms present in the wood (e.g., nematodes, other fungal species). For example, cultures No. H97-14-1A and H97-14-2A (two unidentified fungi) were observed to be parasitizing either another fungal colony, a co-inhabitant in a mixed culture, or themselves (self-parasitism). Self-parasitism has been documented in other wood-decaying fungi (e.g., *Stereum hirustutm*), but the biological significance of this phenomenon is unknown (Rayner and Boddy 1988).

Many of the fungi identified represent soft-rot fungi (Rayner and Boddy 1988) that primarily live off carbohydrates (e.g., hemicellulose, cellulose) found in moist wood or by-products of decay that leach into the wood through the activities of other microorganisms. These fungi may represent a succession of fungi that grow into the wood after gaining entrance in the heartwood and sapwood through the activities of primary wood-decay fungi (e.g., white rot and brown rot fungi), fungal pathogens, animal vectors (e.g., foraging woodpeckers), and/or wood-inhabiting and wood-boring insects in injured living or dead trees. The relative abundance of secondary invaders into the wood and the establishment of a decay community in our samples suggests that the presence of this community also softens wood and facilitates the establishment of nest cavities by woodpeckers. We suggest that initial decay of trees is caused by a basidiomycete, such as *Fomitopsis pinicola*, which sets the stage for secondary invasion by a variety of soft-rot fungi and yeast species. The collective decay caused by this suite of organisms may then facilitate excavation of cavities by woodpeckers. It is possible that the introduction of a variety of fungal organisms, and not just one species, could expedite the use of trees by woodpeckers.

Experimental Tree Inoculations

In 1997 and 1998, we completed inoculation of 650 trees, fulfilling the original experimental design *(table 1)*. Fifteen of the inoculated stands were on Rayonier lands, nine on lands managed by the Department of Natural Resources, four on I. P. Pacific Timberlands Inc. lands, four on Weyerhauser lands, and two on John Hancock Timber Resource Group lands *(fig. 2)*. During the summers of 1997 and 1998, we inoculated 65 clusters of 10 trees each within 34 separate managed forest stands *(table 3)*. A total of 650 trees were inoculated, 330 with viable fungus and 320 trees served as controls and were inoculated with blank dowels. At one stand, we determined that the treatment cluster (T97-04; see Huss and others 1999) was most appropriately classified as a small-diameter tree stand with snags, but that the nearby control cluster (C97-04) contained too few snags. Therefore, two additional small-diameter stands were located at another site with compatible characteristics to complete the pairing for those two mismatched clusters.

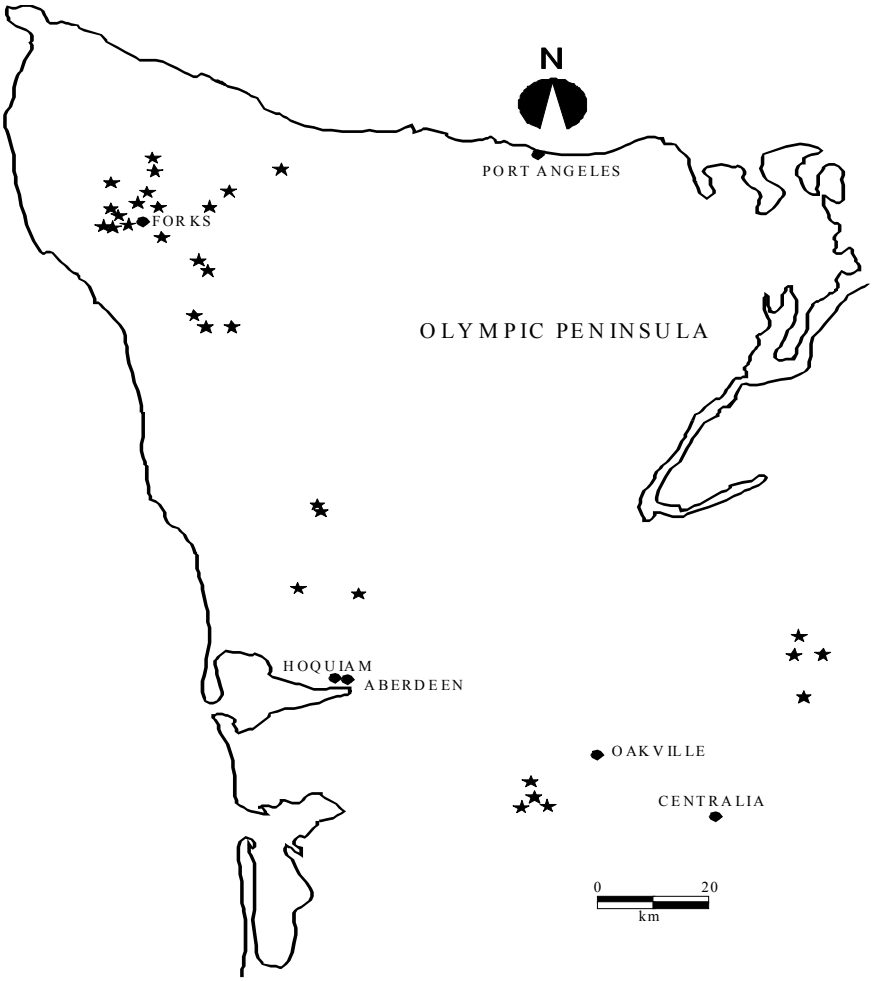

Figure 2—Location of fungal inoculation sites (identified by stars) in western Washington. Sites are on land owned by Rayonier (*N*=15), the State of Washington (managed by the Department of Natural Resources; *N*=9), I. P. Pacific Timberlands Inc. (*N*=4), Weyerhaeuser (*N*=4), and John Hancock Timber Resources Group (*N*=2).

Table 3—*Summary of experimental inoculation treatments and controls completed during 1997 and 1998 in western Washington. Each cluster involves the inoculation of 10 trees.*

Stand classification for experiment	Treatment cluster	Control cluster
Western Hemlock		
Large-diameter trees with snags	4	4
Large-diameter trees with few snags	4	4
Small-diameter trees with snags	4	4
Small-diameter trees without snags	5	4
Douglas-fir		
Large-diameter trees with snags	4	4
Large-diameter trees with few snags	4	4
Small-diameter trees with snags	4	4
Small-diameter trees without snags	4	4

In the process of searching for stands with suitable characteristics, we discovered that the age of a stand is not always directly correlated with mean tree diameter. For example, one stand (T97-05 and C97-05) had relatively small diameter trees, but was 147 yr old. We have classified that stand as a small-diameter tree stand with snags. Despite these exceptions, large diameter/older stands were on average 71 yr old, whereas small diameter/younger stands typically averaged less than 51 yr in age *(table 4)*. To facilitate efficient climbing, workers generally inoculated trees with the largest diameter (usually > 35 cm dbh) in small-diameter classified stands and the relatively small-diameter trees (< 50 cm) in large-diameter classified stands. Despite these opposing biases, inoculated trees in small diameter stands (0 dbh = 39 cm) were clearly smaller than the trees inoculated in large-diameter stands (0 dbh = 45 cm; *table 4)*. These differences in tree diameters between older stands and younger inoculated stands were statistically significant ($P < 0.001$, $t = 4.8$ for comparison of treated plots, $N = 33$; $P < 0.01$, $t = 3.1$ for comparison of control plots, $N = 32$).

Table 4—*Mean characteristics of forest stands inoculated with* Fomitopsis pinicola *in western Washington, 1997 and 1998.*

	Treatment stands			Control stands		
	Mean[1]	**(N)**	**Range**	**Mean[1]**	**(N)**	**Range**
Large diameter/older stands						
DBH (cm)	46.9	(16)	37.7-60.2	44.8	(16)	38.3-61.0
Age (years)	71.4	(16)	49-197	71.4	(16)	49-197
Small diameter/younger stands						
DBH (cm)	38.9	(16)	31.1-49.9	38.9	(16)	30.6-44.8
Age (years)	50.8	(16)	30.0-147.0	51.8	(15)	30.0-147.0

[1]Values were derived from trees treated and may not be representative of average tree characteristics in stand.

Two classes of stands were particularly difficult to locate: larger tree diameter stands without snags and smaller tree-diameter stands with snags. These stand criteria are somewhat contrary to natural succession patterns. Later successional-stage forests generally exhibit some mortality and thus are very likely to contain a conspicuous number of snags. Conversely, early successional-stage forests normally have relatively little tree mortality coupled with the fact that small-diameter snags are often not persistent and fall over after a few years. For this reason, we accepted some stands in these two categories that only marginally fit our original study plan. The most difficult stand criteria to meet were older/large diameter tree stands with no snags. We will examine samples of inoculated trees at 3-yr intervals and determine if cavity-nesting species have colonized these sites.

Establishment of Fomitopsis pinicola *in Inoculated Trees*

One to three years after inoculation of trees with *F. pinicola*, fungal mycelia have been observed in some trees around the entrance of the original inoculation hole created when we introduced the fungus into the wood. The formation of basidiocarps or conks consistent with the morphology of this species was also observed in 2000 *(fig. 3),* emerging from the bark just below the point of initial inoculation. Of 20 randomly selected trees inoculated in 1997 and sampled in 1998, *F. pinicola* (red-belted conk), was retrieved from 14 (70 percent). Of the trees sampled in 1999, 20 inoculated in 1997, and 18 inoculated in 1998, the success rate of retrieving the introduced fungus was 50 percent in both cases. We only took one wood sample from each tree; consequently, even trees from which *F. pinicola* was not recovered, it is likely that this fungus was successfully introduced into at least a few of these and missed by us during the retrieval and culture isolation process. Once it was determined that a tree possessed *F. pinicola*, we tested whether or not the strain recovered matched the strain introduced (i.e., 64A or 36A). Based on vegetative compatibility analysis, 100 percent (N = 33) of the samples collected matched the fungal isolate introduced previously into that tree *(fig. 4)*. These results demonstrate that the fungal inoculations are largely successful (greater than or equal to 50 percent to 70 percent success) in introducing the desired fungus into both western hemlock and Douglas-fir.

Figure 3—Basidiocarp of the red belted conk *(Fomitopsis pinicola)* seen emerging from surface of a living western hemlock tree (T97-01-02) inoculated in 1997 (3-years post treatment).

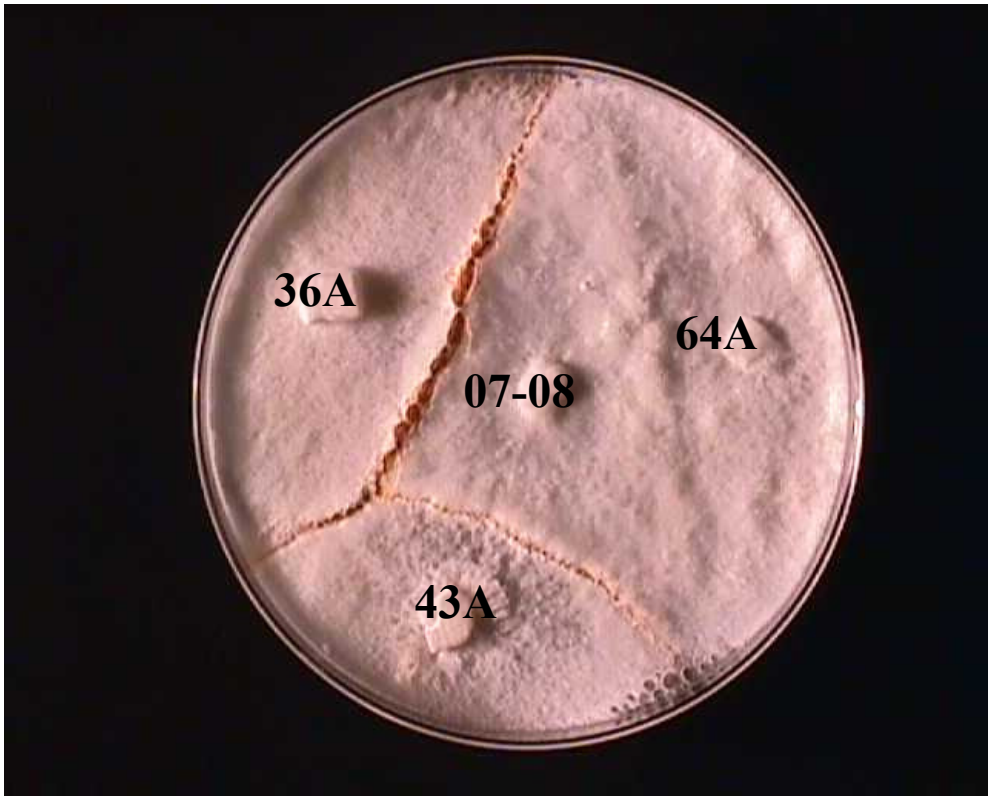

Figure 4—Pairing between isolates recovered from wood in 1998 (central colony) with trees inoculated with *Fomitopsis pincola* (64A) in 1997 with three test strains (peripheral colonies—36A, 43A, and 64A). Isolate 07-08 retrieved from wood is compatible with culture 64A but not with the other two peripheral cultures.

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